(S306N by protein numbering), respectively. These sequences illustrate the existence of a polymorphism. 12 patient DNAs were sequenced, which made it possible to observe a low polymorphism rate between the DNAs tested. For example, comparison of the sequences derived from two individuals, noted 10 and 21, shows the presence of a nucleic acid difference of three bases over the 1617 bases of the gene, which corresponds to a polymorphism rate of 0.19%. Two mutations are located on the sequence of DNA 10 (T671C and G920A) and one on the sequence of DNA 21 (T386C). The sequence of individual 6 is used as the reference. This same analysis at the protein level makes it possible to observe 3 mutated amino acids for the entire envelope comprising, in total, 538 amino acids, i.e. a polymorphism rate of 0.56%. The two mutations of the sequence derived from individual 10 are V224A and S306N, and that of the sequence derived from individual 21 is V128A.

B3 cont

REMARKS

Claims 1-20, 25 and 27-34 are pending.

The attached Appendix includes marked-up copies of each rewritten paragraph (37 C.F.R. §1.121(b)(1)(iii)).

The attached paper copy and computer-readable copy of the Sequence Listing are submitted in compliance with 37 C.F.R. §§1.821-1.825. The contents of the paper copy and the computer-readable copy of the Sequence Listing are the same. No new matter is added. Support for the information provided in the Sequence Listing can be found in the original Sequence Listing and specification at least in Table III and Figs. 1-3.

Early and favorable consideration on the merits is respectfully requested.

Respectfully submitted,

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WPB:PAC/jam

Attachments:
Abstract
Appendix
Sequence Listing (paper and computer-readable copies)

Date: January 6, 2003

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ABSTRACT OF THE DISCLOSURE

A method for detecting the expression of an envelope protein or polypeptide of a human endogenous retrovirus that has the polypeptide sequence of SEQ ID NO:1, a fragment of SEQ ID NO:1 or a sequence having at least 90% identity for every sequence of 20 amino acids with SEQ ID NO:1 or with a fragment of SEQ ID NO:1, by detecting the fusogenic power of the protein or fragment in cells of a cell tissue or cell culture by demonstrating the formation of syncytia. A gene, a nucleic acid or a fragment thereof is used for preparing a therapeutic or prophylactic composition, in particular for treating cancers and for preventing deficiency in placental development.

APPENDIX

Changes to Abstract:

The attached Abstract is added to the specification.

Changes to Specification:

The Sequence Listing is replaced.

Page 1, before line 1, page 1, between lines 34 and 35, page 3, between lines 4 and 5, page 6, between lines 6 and 7, and page 7, before line 1, new paragraph headings are added.

Page 6, lines 27-32:

Examples of polymorphic Env HERV-W sequences are represented in the attached figure 1, the corresponding DNA sequences being represented in figure 2. These figures represent the alignment of protein and nucleic acid sequences obtained by sequencing clones derived from three different individuals. Specifically, figure 1 represents the consensus protein sequence (SEQ ID NO:55) aligned with the three individual protein sequences respectively labeled DNA 6 (SEQ ID NO:57), DNA 10 (SEQ ID NO:59) and DNA 21 (SEQ ID NO:61), and figure 2 represents the corresponding consensus DNA sequence (SEQ ID NO:54) aligned with the three individual DNA sequences respectively labeled DNA 6 (SEQ ID NO:56), DNA 10 (SEQ ID NO:58) and DNA 21 (SEQ ID NO:60).

Page 6, lines 34-39:

Moreover, the polymorphism of the LTR which directs the transcription of the *env* gene located on chromosome 7 was studied. Two groups of 5' LTRs are observed, the nucleic acid sequences of which, obtained by sequencing two clones originating from two different individuals, are represented and aligned in figure 3. Specifically, figure 3 represents the consensus DNA sequence (SEQ ID NO:62) aligned with the two individual DNA sequences respectively labeled LTR6 c1A (SEQ ID NO:63) and LTR21 c1S (SEQ ID NO:64).

Page 18, line 17 to page 19, line 13:

The production of polyclonal and monoclonal antibodies is part of the general knowledge of those skilled in the art. Mention may be made, by way of reference, of Köhler G. and Milstein C. (1975): Continuous culture of fused cells secreting antibody of predefined specificity, Nature 256: 495-497 and Galfre G. et al. (1977) Nature, 266: ~~522-550~~550-552, for the production of monoclonal antibodies, and Roda A., Bolelli G.F.: Production of high-titer antibody to bile acids, Journal of Steroid Biochemistry, Vol. 13, pp 449-454 (1980), for the production of polyclonal antibodies. For the production of monoclonal antibodies, an immunogen may be coupled to Keyhole Limpet Hemocyanin (KLH peptide) as a support for the immunization, or to serum albumin (SA peptide). The animals are given an injection of immunogen using complete Freund's adjuvant. The sera and the hybridoma culture supernatants derived from the immunized animals are analyzed for their specificity and their selectivity, using conventional techniques, such as for example ELISA or Western blot assays. The hybridomas producing the most specific and the most sensitive antibodies are selected. Monoclonal antibodies may also be produced *in vitro* by cell culture of the hybridomas produced or by recovery of ascites fluid, after intraperitoneal injection of the hybridomas into mice. Whatever the method of production, by supernatant or by ascites, the antibodies are then purified. The purification methods used are essentially ion-exchange gel filtration and exclusion chromatography or immunoprecipitation. A number of antibodies sufficient to identify the most effective ones are screened in functional assays. The *in vitro* production of antibodies, of antibody fragments or of antibody derivatives, such as chimeric antibodies produced by genetic engineering, is well known to those skilled in the art.

Page 20, lines 10-21:

The expression "element which ensures the expression *in vivo* of said gene" refers in particular to the elements required to ensure its expression after it has been transferred into a

target cell. They are, in particular, the promoter sequences and/or the regulatory sequences which are effective in said cell and, optionally, the sequences required to allow the expression, at their surface ~~[sic]~~, of an inhibitory polypeptide or molecule, as mentioned above. The promoter used may be a viral, ubiquitous or tissue-specific promoter or a synthetic promoter. Examples of such promoters have been described previously.

Page 25, line 4 to page 26, line 11:

In order to study the polymorphism of the coding region of the envelope and of the associated 5' LTR U3 promoter region, located on chromosome 7, amplification specific for a 10 kb fragment is carried out using a pair of specific primers. In fact, given that the HERV-W family comprises many noncoding copies and in particular a considerable number of LTRs, this strategy makes it possible to specifically and jointly amplify the *env* region and its promoter sequence (5' LTR) located upstream, exclusively on chromosome 7. For this, use is made of a primer U6198 (SEQ ID NO:29) which hybridizes on a specific sequence located upstream of 5' LTR on chromosome 7, and a primer L6186 (SEQ ID NO:30) which hybridizes in an overlapping manner on the 3' LTR U5 region and the adjacent cellular gene, on this same chromosome. Long distance PCR (or LD-PCR) is carried out under the following conditions, 1 × 5 min at 94°C, 10 × (10 sec at 94°C, 30 sec at 55°C, 8 min at 68°C), 25 × (10 sec at 94°C, 30 sec at 55°C, 8 min at 68°C + 10 sec/cycle), 1 × 7 min at 68°C, in the presence of amplification buffer (50 mM Tris HCL, pH 9.0, at 25°C, 15 mM $(NH_4)_2SO_4$, 0.1% Triton X-100); 1.5 mM $MgCl_2$, 0.25 mM of each dNTP, 330 nM of each primer (U6198 and L6186), 1U of DNA polymerase and also 200 ng of matrix (genomic DNA) in a final volume of 50 ml.

A nested "env" PCR and also a nested "LTR" PCR are carried out using this diluted 10 kb PCR product, in order to objectify the presence or absence of a polymorphism of these two

regions. The dilution allows specific amplification from the LD-PCR product and not from the starting genomic material. The nested "env" PCR is carried out using the U6189 (SEQ ID NO:31) and L6186 (SEQ ID NO:30) primers, the U6189 ~~[sic]~~ primer being that used for the LD-PCR, the U6189 primer being located upstream of the env ATG. The 5' LTR U3 region is amplified with the U6460 (SEQ ID NO:32) and L5643 (SEQ ID NO:33) pair of primers. The U6460 primer hybridizes upstream of the 5' LTR, while the L5643 primer hybridizes in the R domain of the 5' LTR. The nested PCRs are carried out under the following conditions, 1 × 5 min at 94°C, 30 × (1 min at 94°C, 1 min at 53°C, 3 min at 72°C), 1 × 7 min at 72°C, in the presence of amplification buffer (10 mM Tris HCL, pH 8.3, 50 mM KCl), 1.5 mM $MgCl_2$, 0.25 mM of each dNTP, 330 nM of each primer, 1.25U of DNA polymerase and an aliquot of the LD-PCR amplification product, in a final volume of 50 ml.

Page 26, line 22 to page 27, line 20:

Polymorphism of the *env* gene: the use of 20 primers (10 even sense primers: 6302 to 6320, SEQ ID NOs: 34, 36, 38, 40, 42, 44, 46, 48, 50 and 52, and 10 odd antisense primers: 6303 to 6321, SEQ ID NOs: 35, 37, 39, 41, 43, 45, 47, 49, 51 and 53) makes it possible to sequence the coding region of the envelope using the nested envelope PCR product. These primers may also be used for analysis of the polymorphism by SSCP. By way of example, the sequences of the envelope genes of three healthy donors labeled D6, D10 and D21, are illustrated in figure 2. These sequences show the existence of a low polymorphism rate. If the envelope sequence of donor D6 is used as an arbitrary reference, the sequence of the envelope of donor D21 has a mutation at position 386 (T386C), the replacement of the thymine with cytosine inducing an amino acid change of valine to alanine (V128A by protein numbering). Similarly, the sequence of the envelope gene of donor D10 has two mutations relative to the sequence of donor D6, at position 671 (T671C) and 920 (G920A), inducing two amino acid changes, from valine to alanine (V224A by protein numbering) and from serine to asparagine

(S306N by protein numbering), respectively. These sequences illustrate the existence of a polymorphism. 12 patient DNAs were sequenced, which made it possible to observe a low polymorphism rate between the DNAs tested. For example, comparison of the sequences derived from two individuals, noted 10 and 21, shows the presence of a nucleic acid difference of three bases over the 1617 bases of the gene, which corresponds to a polymorphism rate of 0.19%. Two mutations are located on the sequence of DNA 10 (T671C and G920A) and one on the sequence of DNA 21 (T386C). The sequence of individual 6 is used as the reference. This same analysis at the protein level makes it possible to observe 3 mutated amino acids for the entire envelope comprising, in total, 538 amino acids, i.e. a polymorphism rate of 0.56%. The two mutations of the sequence derived from individual 10 are V224A and S306N, and that of the sequence derived from individual 21 is V128A.

APPENDIX

Changes to Claims:

Claim 21-24 and 26 are canceled.

The following are marked-up versions of the amended claims:

3. (Amended) The method as claimed in claim 1 ~~or 2~~, characterized in that the protein is encoded by the *env*gene of the HERV-W endogenous retrovirus.

9. (Amended) The method as claimed in claim 1 ~~any one of the preceding claims~~, characterized in that the cells of said tissue or of said cell culture are chosen from bone cells, muscle cells, placenta cells, endothelial cells, in particular of blood vessels, epithelial cells, glial cells and tumor cells or cells derived from tumor cell lines.

10. (Amended) The method as claimed in claim 1 ~~any one of the preceding claims~~, characterized in that the detection of the fusogenic power of said protein consists in: obtaining a vector for expression of said protein, based on which the expression of the protein or of its gene is under the control of a promoter, preferably a strong promoter, transfecting cells with the vector obtained, so as to obtain producer cells expressing, at their surface, said protein, and observing the formation of syncytia or the absence of formation of syncytia.

11. (Amended) The method as claimed in claim 1 ~~any one of the preceding claims~~, characterized in that the detection of the fusogenic power of the protein consists in: obtaining a vector for expression of said protein, based on which the expression of the protein or of its gene is under the control of a promoter, preferably a strong promoter, transfecting cells with the vector obtained, so as to obtain producer cells expressing, at their surface, said protein, coculturing naïve indicator cells, expressing, at their surface, a receptor for said protein, in the presence of said producer cells, and observing the formation of syncytia or the absence of formation of syncytia.

15. (Amended) The use as claimed in claim 12, ~~13 or 14,~~ characterized in that the composition is intended for treatment by gene therapy.

16. (Amended) A therapeutic or prophylactic composition comprising a fragment of gene or of nucleic acid coding for a polypeptide as defined in claim 7 ~~or 8~~.

18. (Amended) An expression vector comprising at least one fragment of gene or of nucleic acid coding for a polypeptide as defined in claim 7 ~~or 8~~, and elements required for its expression in a host cell.

25. (Amended) A gene therapy vector comprising a polypeptide as defined in claim 7 ~~or 8~~.

27. (Amended) A therapeutic composition comprising, inter alia, a therapy vector as defined in claim 25 ~~either of claims 25 and 26~~, and an antisense nucleic acid sequence or oligonucleotide.

28. (Amended) A therapeutic composition comprising, inter alia, a therapy vector as defined in claim 25 ~~either of claims 25 and 26~~, and a gene of therapeutic interest.

29. (Amended) A therapeutic composition comprising, inter alia, a cellular vector comprising a cell expressing a protein or a polypeptide as defined in claim 1 ~~any one of claims 1 to 9~~.

30. (Amended) A method for selecting medicinal substances or drugs, or gene/prodrug systems, capable of having a qualitative and/or quantitative effect on the fusogenic power of a protein or of a polypeptide as defined in claim 1 ~~any one of claims 1 to 8~~, according to which said medicinal substance or drug, or said gene/prodrug system, is brought into contact with cells of a cell culture expressing said protein or said polypeptide, and a regression or a disappearance of the formation of syncytia is observed.

31. (Amended) A therapeutic composition comprising, inter alia, an antisense nucleic acid sequence or oligonucleotide capable of hybridizing to a gene or a fragment of

gene, or to a nucleic acid or fragment of nucleic acid, coding for a protein or a polypeptide as defined claim 1~~in any one of claims 1 to 8~~.

34. (Amended) The use as claimed in claim 32~~or 33~~, characterized in that the [lacuna] a ligand chosen from a monoclonal antibody, a polyclonal antibody, a transmembrane antibody or a fragment of said antibodies, and an inhibitory molecule, said ligand being specific for the receptor of the protein defined in SEQ ID No. 1.